A Microbial Perspective:
Exploring the Impacts of Invasive Himalayan Blackberry on Soil Bacterial Diversity in BC's Temperate Rainforests

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Introduction

Invasive plant species have continued to spread globally at unprecedented rates, negatively impacting ecosystem dynamics. Notably, previous studies on invasive plants have emphasized their influence on native plant and animal species, opposed to soil microbiology. However, understanding their effects on microbes is crucial, as microbes play significant roles in the ecosystems they inhabit.

When considering how invasive plants effect bacterial communities specifically, Zhang et al. (2024) found that the presence of an invasive species known as *Spartina alterniflora* decreased the diversity of soil bacteria. Although, other studies have found contradictory results, and so the exact effect invasive plants have on microbial communities remains unclear. Therefore, this project sought to strengthen our understanding on such interactions.

Purpose & Hypothesis

Despite Himalayan blackberry being the most common invasive plant in southwest BC (Gaire et al., 2015), there is no existing literature on its soil microbial effects. Given the global significance of BC's coastal biodiversity (MacDuffee, 2024), understanding the impacts of this invasive species is pivotal. Thus, the objectives of this research project were as follows:

- **To learn more about the overall interconnectivity between invasive plants and soil bacteria**
- **To explore how invasive Himalayan blackberry (*Rubus armeniacus*) affects soil bacterial abundance and diversity, compared to native salal (*Gaultheria shallon*)**

The hypothesis for this experiment was that there would be a difference in bacterial diversity in soil sampled near invasive Himalayan blackberry, compared to soil sampled near native salal. Specifically, it was predicted that invaded soil samples would have lower bacterial diversity compared to that of non-invaded soils.

Methodology

A total of twenty soil cores were collected from five sites across Mundy Park and Riverview Forest, in Coquitlam. At each site, four cores were collected; two from under invasive Himalayan blackberry, and two from beneath native salal.

The cores were obtained using a 12-inch sample probe, and transported in containers lined with sterilized tin-foil. Each core was divided into top, middle, and bottom sections. From each section, 2.5 g of soil was mixed with 37.5 mL of distilled water, and centrifuged to isolate the bacterial cells. The resulting supernatant was further diluted, before 0.5 mL was plated on nutrient agar. Control plates were prepared using 0.5 mL of distilled water. The pH and salinity of the supernatant of the lowest soil levels were also tested.

The 60 total plates were incubated at room temperature for up to 3 days. Bacterial relative abundance (CFU's/g of soil) and the reciprocal of Shannon Diversity Index were calculated. These values were then compared using one-way ANOVA and independent sample t-tests.

Figures 1-3: From left to right; Himalayan blackberry in site #2, salal in site #2, and plates from site #5 depicting differences in bacterial growth between invaded and non-invaded soils across the three soil core depths.

Results

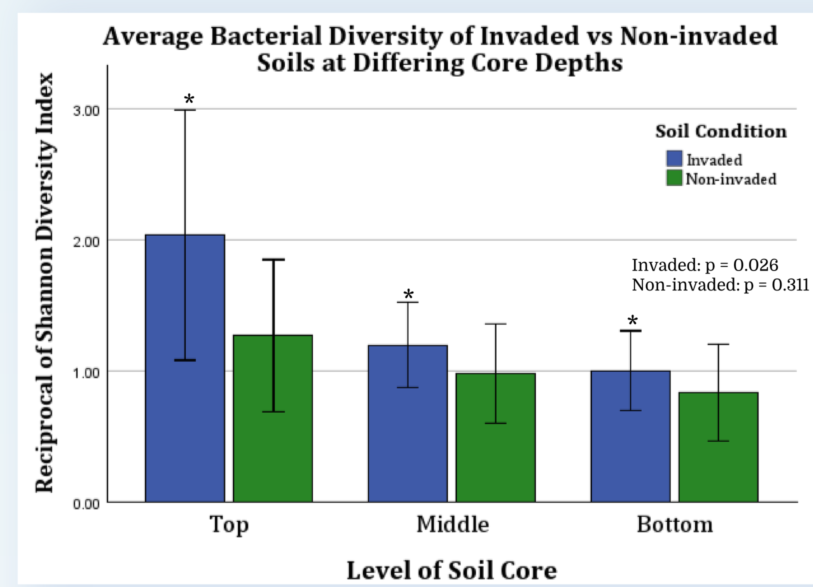

Figure 4: Average reciprocal Shannon Diversity Index for invaded (blue) and non-invaded (green) soils across three soil core depths. *Only invaded soils had a significant difference in diversity across the levels ($p = 0.026$). Error bars represent 95% CI.

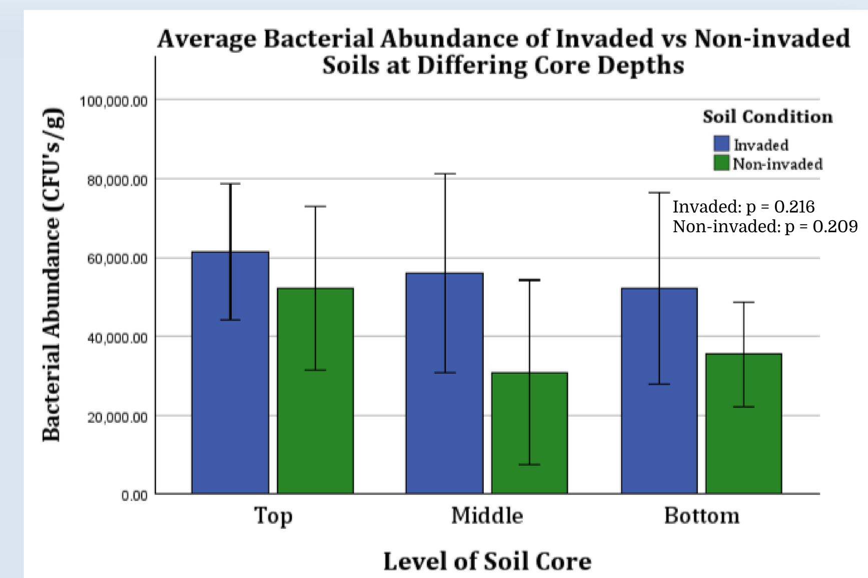

Figure 5: Average bacterial abundance (CFU's/g of soil) in invaded versus non-invaded soils across the three different soil core depths. Neither soil condition had significant differences in abundance within the different levels. Error bars represent 95% CI.

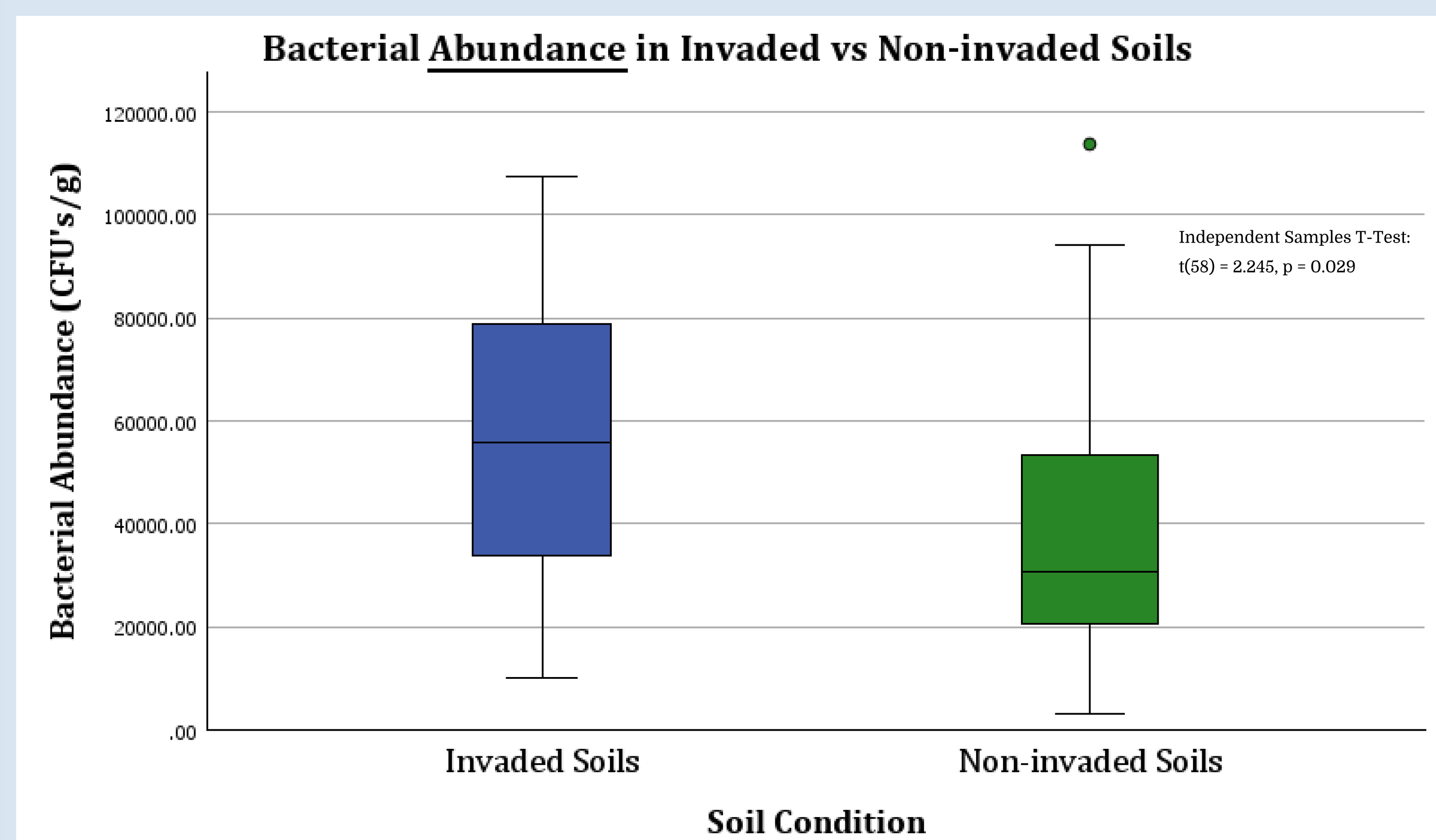

Figure 6: Bacterial abundance (CFU's/g) in invaded (blue) vs non-invaded (green) soils. The average abundance for invaded soil samples was 56,523 CFU's/g, and 39,492 CFU's/g for non-invaded soils. Non-invaded soils had an outlying abundance value of 113,644 CFU's/g. Invaded soils had a significantly higher bacterial abundance ($p = 0.029$).

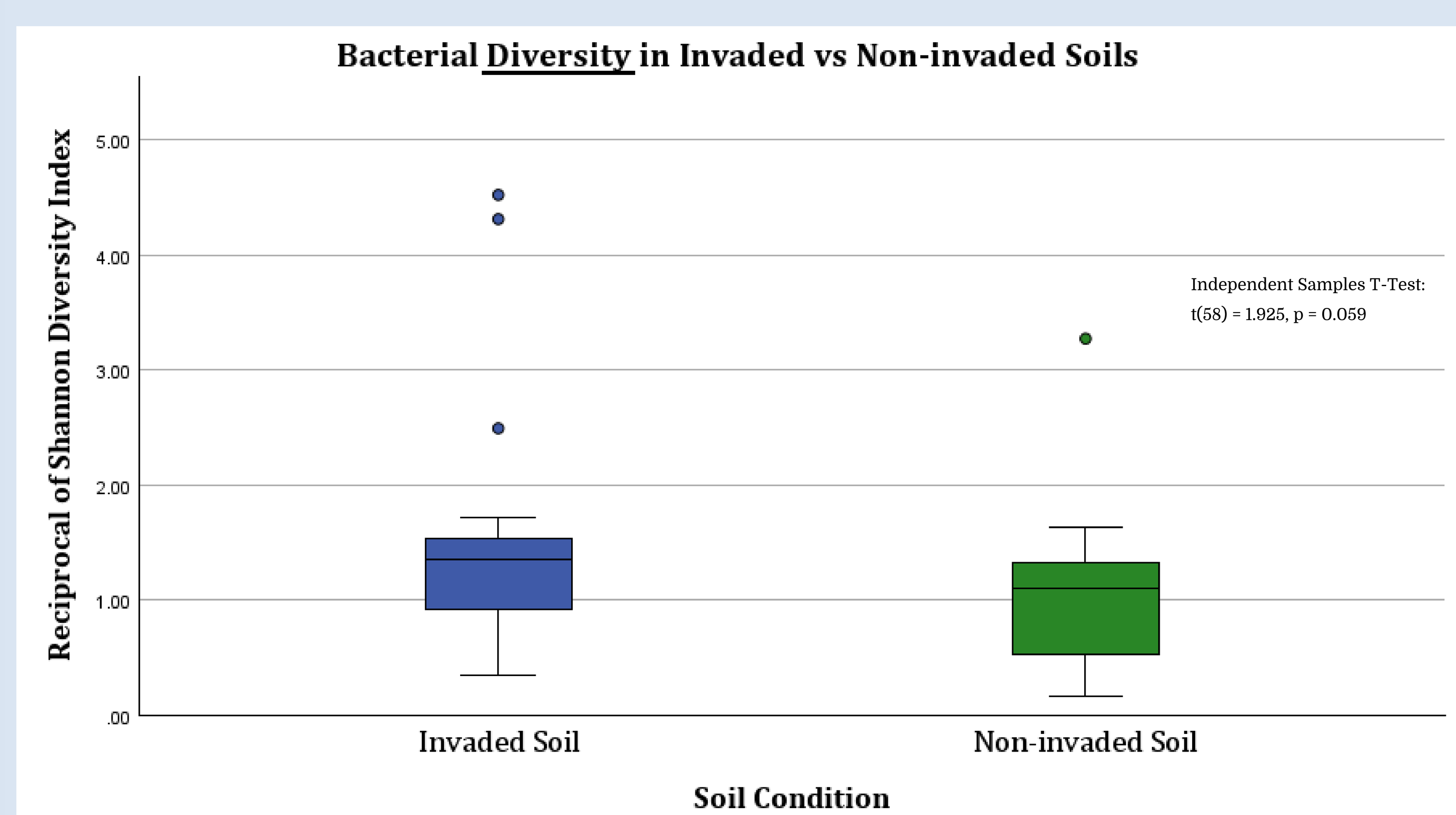

Figure 7: Bacterial diversity values (the reciprocal of the Shannon Diversity Index) for invaded vs non-invaded soils. Average bacterial diversity for invaded soil samples was 1.413, while the average for the non-invaded soils was 1.017. Invaded soils had more outlying diversity values than non-invaded soil samples. The standard two-sided p value was insignificant ($p = 0.059$).

Results

Upon examining the effects soil depth had on bacterial diversity, ANOVA results indicated a significant difference within invaded soils ($F(2) = 4.194$, $p = 0.026$), but not within non-invaded soils (Figure 4). Additionally, bacterial abundance had no significant difference across the three soil depths in either invaded ($F(2) = 0.216$, $p = 0.807$) or non-invaded soils ($F(2) = 1.660$, $p = 0.209$) (Figure 5).

The independent sample t-tests indicated a significant difference in the bacterial abundance ($t(58) = 2.245$, $p = 0.029$) between invaded and non-invaded soil conditions (Figure 6).

Visually, plates from invaded soil samples appeared to have greater diversity compared to non-invaded samples (Figure 3). However, the two-sided p value indicated insignificance ($p = 0.059$) (Figure 7). Soil pH and salinity also did not differ significantly between the two soil conditions ($p > 0.05$).

Discussion

Only invaded soils illustrated differences in bacterial diversity across the three soil depths, with the highest diversity at the surface. In general, surface soil typically has greater bacterial diversity due to increased exposure to air and organic matter (Naylor, 2022). Thus, it was unexpected that only invaded soils showed an increase in diversity.

While overall bacterial abundance was significantly higher in invaded soils compared to non-invaded soils, bacterial diversity did not differ significantly between the two soil types. Although the one-sided p value for diversity suggested a directional difference ($p = 0.030$), the standard two-sided p-value indicated insignificance ($p = 0.059$). Therefore, the results of this study do not support the hypothesis or prediction.

The insignificant diversity result, despite noticeable differences between plates, could be due to data collection limitations. Specifically, only colony morphology was used to categorize colonies; using cell morphology and DNA sequencing may have better reflected bacterial diversity differences between the samples.

Invasive plants are known to alter soil chemical composition via allelochemicals, in turn influencing soil microbial communities (Torres et al., 2021). However, the invaded and non-invaded soils had no difference in pH and salinity. This was unexpected - even without considering the effects of different plant species, the abiotic characteristics would in theory change in different site locations.

In summary, this study found that Himalayan blackberry increases bacterial abundance in the surrounding soil compared to salal, yet it has no significant impact on overall soil bacterial diversity. This could mean Himalayan blackberry provides favorable conditions for specific types of bacteria, by altering soil characteristics other than the pH and salinity via allelochemicals. Future studies will be needed to confirm Himalayan blackberry's influence on soil bacterial communities in BC's temperate rainforests, and in turn, how the ecosystems they inhabit are impacted as a whole.

References

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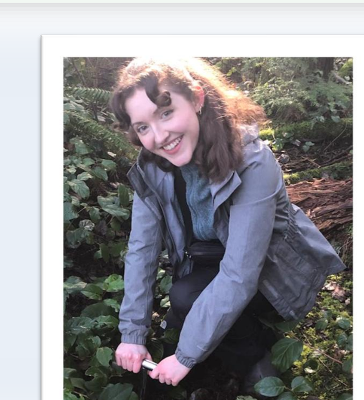
Invasive species hate to see me coming!



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